

Docket No. 00600.449US1  
WD # 435536.wpd



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Clean Version of Pending Claims

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TRANSGENIC CIRCULATING ENDOTHELIAL CELLS

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Serial No.: 09/865,022

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1. A process for expanding the population of endothelial cells obtained from peripheral blood comprising culturing, in contact with a collagen I-coated surface, buffy coat cells obtained from peripheral mammalian blood in the presence of a cell culture medium containing an effective amount of vascular endothelial growth factor (VEGF), and which medium is free of bovine brain extract, so as to expand the population of endothelial cells in said buffy coat cells.
  2. The process of claim 1 wherein the blood is human blood.
  3. The process of claim 1 wherein said cell culture medium comprises heparin, dextran sulfate or mixtures thereof.
  4. The process of claim 1 wherein the buffy coat cells are obtained by washing cells from a buffy coat layer obtained from human blood in cell culture medium comprising 20% human male serum.
  5. The process of claim 1 wherein the cell culture medium comprises human basic fibroblast growth factor.
  6. The process of claim 1 or 5 wherein the cell culture medium comprises insulin-like growth factor.
  7. The process of claim 1 or 5 wherein the cell culture medium contains human epidermal growth factor.



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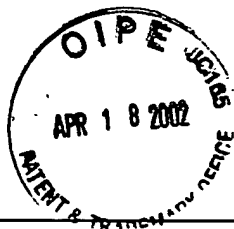
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8. The process of claim 1 wherein the cell culture medium comprises about 0.5-10 vol-% fetal bovine serum and about 95-99.5 vol-% of a cell culture medium.
9. The process of claim 1 wherein the cultured cells are trypsinized at about  $10^3$ -fold expansion, collected by centrifugation, resuspended in cell culture medium, and subjected to continued culture in contact with a fibronectin/gelatin- coated surface.
10. The process of claim 1 wherein the cultured cells are subjected to cryopreservation.
11. The process of claim 10 wherein the cells are frozen in a cryopreservation medium comprising fetal calf serum containing an effective amount of dimethylsulfoxide.
12. The process of claim 10 or 11 wherein the cryopreserved cells are thawed and culturing is resumed in said cell culture medium.
13. The process of claim 1 wherein the expanded population comprises microvascular endothelial cells.
14. The process of claim 13 wherein the microvascular endothelial cells are CD34<sup>+</sup>, CD36<sup>+</sup> and express the P1H1 antigen.
15. A transgenic mammalian endothelial cell comprising an isolated DNA sequence encoding a recombinant Factor VIII protein.
16. The transgenic endothelial cell of claim 15 wherein the Factor VIII protein is a hybrid human/porcine protein.

17. A transgenic endothelial cell which is prepared by a process comprising stably transforming a population of circulating human endothelial cells outgrown from blood with a vector comprising an isolated DNA sequence encoding a preselected protein operably linked to a promoter functional in human endothelial cells.
18. The transgenic endothelial cell of claim 17 wherein the DNA sequence encodes a Factor VIII protein.
19. The transgenic endothelial cell of claim 17 wherein the DNA sequence comprises SEQ ID NO:1 or SEQ ID NO:2.
20. The transgenic endothelial cell of claim 18 wherein the Factor VIII protein is a hybrid human/porcine protein.
21. The transgenic endothelial cell of claim 18 wherein the Factor VIII protein is a chimeric human protein.
22. The transgenic endothelial cell of claim 17 wherein the DNA sequence further comprises a selectable marker gene or a reporter gene.
23. The transgenic endothelial cell of claim 17 which is prepared by lipofection.
24. A pharmaceutical composition comprising a population of the transgenic endothelial cells of claim 15 or 17 in combination with a pharmaceutically acceptable carrier.



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25. A method of treating hemophilia comprising introducing an amount of the endothelial cells of claim 15 or 17 into the bloodstream of a mammal afflicted with hemophilia so that an effective amount of a Factor VIII protein is secreted into the bloodstream of said mammal.
26. The method of claim 25 wherein the mammal is a human.
27. An implantable medical prosthetic device comprising a surface coated with the endothelial cells of claim 17 wherein the protein is expressed in an amount effective to increase the biocompatibility of said device upon implantation into a mammal.
28. The device of claim 27 which comprises a plastic surface, a metal surface or a laminate surface.
29. The device of claim 28 which is a vascular graft.
30. The device of claim 28 which is a shunt or a stent.
31. The device of claim 28 which is a heart valve.
32. The device of claim 28 which is a controlled drug release depot.
33. A diagnostic method comprising:  
detecting or determining whether an expanded population of endothelial cells obtained from a test mammal has an acquired or genetic indication or disease relative to a control expanded population of endothelial cells obtained from a mammal not at risk of the acquired or genetic indication or disease, wherein the expanded cells are obtained by culturing, in contact

with a collagen I-coated surface, buffy coat cells obtained from peripheral mammalian blood in the presence of a cell culture medium containing an effective amount of vascular endothelial growth factor (VEGF), which medium is free of bovine brain extract, so as to yield an expanded population of endothelial cells.

34. The method of claim 33 wherein the indication or disease is a clotting disorder.
35. The method of claim 33 wherein the indication or disease is associated with a reduction in the activity of an enzyme.
36. The method of claim 33 wherein the indication or disease is acquired.
37. (New) A transgenic endothelial cell comprising a vector comprising an isolated DNA sequence encoding a preselected protein operably linked to a promoter functional in human endothelial cells, which transgenic endothelial cell is prepared by introducing the vector into an expanded population of endothelial cells prepared by culturing, in contact with a collagen I-coated surface, buffy coat cells obtained from peripheral mammalian blood in the presence of a cell culture medium containing an effective amount of vascular endothelial growth factor (VEGF), and which medium is free of bovine brain extract.
38. (New) The transgenic endothelial cell of claim 37 wherein the DNA sequence encodes a Factor VIII protein.
39. (New) The transgenic endothelial cell of claim 37 wherein the DNA sequence comprises SEQ ID NO:1 or SEQ ID NO:2.

40. (New) The transgenic endothelial cell of claim 38 wherein the Factor VIII protein is a hybrid human/porcine protein.
41. (New) The transgenic endothelial cell of claim 38 wherein the Factor VIII protein is a chimeric human protein.
42. (New) The transgenic endothelial cell of claim 37 wherein the DNA sequence further comprises a selectable marker gene or a reporter gene.
43. (New) The transgenic endothelial cell of claim 37 which is prepared by lipofection.
44. (New) A population of expanded endothelial cells prepared by the method of claim 1.